SECTION ON MICROBIOLOGY*

ABSTRACTS OF PAPERS

Presented at the Meeting, December 21, 1955 at The New York Academy of Medicine

The Relation of a Circulating Endogenous Pyrogen to the Cause of Experimental Fever

ELISHA ATKINS

Yale University School of Medicine, New Haven, Conn.

Despite extensive studies on the physiology of temperature regulation the stimuli which act upon the hypothalamus to cause the fevers of disease remain unknown. The recent recovery of a pyrogenic substance from leukocytes by Bennett and Beeson has added support to the hypothesis that fever may be due to cellular injury in diseases involving inflammation. However, no such substance has hitherto been detected in the circulation.

The present studies deal with the presence of an endogenous pyrogen in the blood stream of rabbits made febrile by the injection of typhoid vaccine. Three types of donor animals were employed: 1) unsensitized donors, not previously challenged with vaccine; 2) "sensitized" donors which had received several previous injections spaced at intervals to obviate the development of tolerance; and 3) donors made pyrogen-tolerant by daily injections of vaccine for a period of a week or more.

The donors were given a standard inoculation of vaccine. Their sera were then tested for pyrogenic activity in both normal and pyrogen-tolerant recipients. With this method of passive transfer the amount of circulating pyrogen in the donor animals could be measured at various intervals after inoculation. By comparison of the responses of the normal and tolerant recipients to each sample of serum it was further possible to differentiate the pyrogen present into exogenous (injected) and endogenous types. Endogenous pyrogen, like the leukocytic pyrogen described by Bennett and Beeson, was found to be as active in tolerant as in normal recipients. On the other hand, the pyrogenic effect of the uncleared vaccine, present in donor serum obtained shortly after injection, was almost completely inhibited in the tolerant recipients.

Characteristic patterns of pyrogenic activity were found in the sera of the three classes of donors following inoculation of the vaccine. In sensitized donors, the clearance of the injected pyrogen was complete before the appearance of the endogenous form. In unsensitized donors, on the other hand, the endogenous pyrogen appeared before their circulations were cleared of the vaccine, resulting in a persistent level of pyrogenic activity throughout. Finally, in tolerant donors, no transferable pyrogen was found at any of the tested intervals. The lack of detectable endogenous pyrogen in this latter group parallels the marked reduction in the febrile response of tolerant animals and has been attributed to the very

* Maclyn McCarty, *Chairman;* A. M. Pappenheimer, Jr., *Secretary.*

rapid clearance of injected vaccine by cells of the reticulo-endothelial system. Because of this, cellular damage, presumably the source of endogenous pyrogen, is believed to be minimized. In conformity with this concept is the finding that endogenous pyrogen reappeared in the sera of tolerant donors given an injection of vaccine after R-E blockade had been established with Thorotrast. In this regard Beeson has shown that the febrile response of tolerant donors to injected pyrogens is restored after Thorotrast. The association of detectable levels of endogenous pyrogen with the augmented febrile response supports the hypothesis that fever is due to the presence of endogenous pyrogen in the circulation.

A close correlation of the duration of fever in both unsensitized and sensitized donors with the presence of endogenous pyrogen in their sera similarly suggests such a causal relationship.

In further preliminary studies, fevers have been induced in rabbits with intrapharyngeal inoculations of group A hemolytic streptococci and intravenous injections of the viruses of influenza A, PR8 strain, and Newcastle disease (NDV). Sera transferred from these donors at the height of their fever several hours later caused characteristic pyrogenic responses in normal and virus-tolerant recipients.

The presence of a circulating endogenous pyrogen in these different forms of experimental fever is indicative of a common pathogenetic mechanism. The similarity of the properties thus far obtained of endogenous and leukocytic pyrogen suggests that the former may be a product of cellular injury. In addition, the rapid onset of fever following the injection of the endogenous substance suggests that it may well be the factor which acts upon the hypothalamus to cause fever.

*Studies of the Febrile Response to Acute Bacterial Infection and Bacterial Pyrogens**

IVAN L. BENNETT, JR.** and WILLIS R. KEENE***

Biological Division of the Department of Medicine, Johns Hopkins University, Baltimore, Maryland

Although some bacteria[1] and a few viruses[2] will provoke high fever when injected into man or experimental animals, many pathogenic microorganisms possess no demonstrable pyrogen, and fever, of course, accompanies many diseases that do not involve any known infectious agent. Because the obvious common denominator of disease is injury to cells, the hypothesis most commonly offered states that fever results from the action of products of tissue damage upon the cerebral centers governing body heat.[3] It is the purpose of this presentation to review briefly some of a series of experimental investigations that bear on this hypothesis.

At the outset, in the study of tissue substances which may produce fever, it is imperative to avoid contamination of test-materials with ordinary bacterial pyrogens. These substances are complex polysaccharides, the endotoxins or somatic antigens characteristic of gram-negative bacteria.[1, 4] They are active in small amounts and are able to withstand autoclaving for several hours without appreciable loss of potency. Because bacterial pyrogens are convenient agents for the production of experimental fever, their action has been studied extensively. Some of the facts which have been learned about the febrile response to injection of bacterial pyrogens are important to an understanding of the problem of studying products of tissue injury as possible factors in the pathogenesis of the fever of disease:

1. The injection of bacterial pyrogens directly into the blood stream of man or experimental animals is followed by a time lag or "latent" period before abrupt rise in body temperature occurs. In the rabbit, this delay amounts to 20-30 minutes; in man, it may be as long as 90 minutes. This lag-period is usually interpreted as indicating that bacterial pyrogens act *indirectly* to cause fever, that some preliminary reaction in the circulating blood or other tissues of the host is necessary to trigger the final stimulus to the central nervous system.[5, 6]

2. The febrile response of the rabbit to intravenous injection of bacterial pyrogen is typically biphasic with an initial rise and fall and a secondary spike.[7]

3. With daily injections of bacterial pyrogen, rabbits (or human subjects) develop a state of resistance to the fever-producing effect of these substances.[5] This "tolerance" is non-specific and is effective against pyrogens from any species of bacteria. It disappears rapidly when daily injections are stopped. The mechanism of pyrogen tolerance is incompletely understood but the observation by Beeson[8] that it is abolished by injection of particulate materials such as Thorotrast has led to the suggestion that it is a non-specific increase in the ability of cells of the so-called reticulo-endothelial system to remove pyrogen from the circulation and prevent its injurious effects. The recent observation of Braude[9] that the degree of tolerance is proportional to the speed with which labelled pyrogen is cleared from the blood stream supports this idea strongly. Humoral factors probably play some part in tolerance also.[7]

In the experiments to be described pyrogen contamination was avoided by sterilizing glassware, needles, etc., at 170°C. for two hours and by testing all solutions and reagents by injection into rabbits before use.

* The investigations that form the basis for this report have been supported by the U. S. Public Health Service, including Grant A-660 of the National Institute of Arthritis and Metabolic Diseases.

** Associate Professor of Medicine.

*** Student Fellow, National Foundation for Infantile Paralysis.

If injured tissues release a substance or substances capable of producing fever, it might be supposed that normal tissues contain fever-promoting substances. Consequently a survey of normal tissues and cells of the rabbit for such a substance was carried out.[10] Normal tissues, including suspensions of macrophages and lymphocytes, were ground in various ways and saline extracts or suspensions were tested by intravenous, intramuscular, intraperitoneal and intrapleural injection into normal rabbits. Similarly, tissues were allowed to undergo sterile autolysis before preparation of extracts or suspensions for testing. Erythrocytes were damaged *in vitro* and reinjected and intravascular hemolysis was produced by injection of distilled water or saponin. Finally, rabbits were subjected to a series of operative procedures resulting in infarction of heart, lung, spleen, or kidney, their temperatures being followed carefully over 21 days postoperatively. None of the tissue preparations or other procedures resulted in fever in test animals.

Saline extracts of acute inflammatory lesions such as the Arthus reaction in rabbits were found to contain a fever-producing substance.[10] The febrile response of normal rabbits to injection of these extracts was monophasic and of brief (three to four hours) duration and the ability of extracts to produce fever was destroyed by heating at 90°C. for 30 minutes, evidence that bacterial contamination was not a factor.

It was finally shown that saline extracts of polymorphonuclear leukocytes collected from saline-induced peritoneal exudates in rabbits or from peripheral blood produced a similar monophasic febrile response as did the cell-free fluid of sterile peritoneal exudates.[11] However, saline injected into the peritoneal cavity of rabbits made neutropenic by nitrogen mustard resulted in the appearance of a fever-producing substance in peritoneal fluids containing no polymorphonuclear cells. The ability of these fluids and extracts to produce fever was destroyed by heating at 90°C. for 30 minutes.

The nature of this fever-producing substance is not yet clear. It is destroyed at 85°C. at pH 7.2 and at 70°C. at pH 4.5. It is non-dialyzable and its activity is uninfluenced by digestion with trypsin, chymotrypsin or ribonuclease. Daily injections produce no tolerance and the ability of rabbits to respond to it is uninfluenced by prior injection of Thorotrast. Present efforts are directed toward fractionating these materials by zone electrophoresis but results to date have not been definitive. The finding of this substance in sterile peritoneal fluids collected from neutropenic animals indicates that the polymorphonuclear leukocyte is not its only source; however, a recently completed survey of extracts of tissues of leukopenic animals again failed to reveal another origin.[12] A similar, perhaps the same, fever-promoting substance has been extracted regularly from the tissues of anaphylactically sensitized animals made febrile by reinjection of antigen.[13]

Peritoneal exudate and thoracic duct lymph collected from rabbits with Type I pneumococcal peritonitis also contain a heat-labile, fever-producing substance with properties similar to those already described.[14] This substance appears when animals develop fever several hours after inoculation of bacteria into the peritoneum and is no longer demonstrable after animals become afebrile as a result of treatment with penicillin. Its presence in thoracic duct lymph, of course, indicates its access to the venous circulation but extensive efforts to detect the fever-promoting factor in peripheral blood have thus far failed. The pneumococcus is not a pyrogen-producing organism. The course of events described in pneumococcal infections holds for both normal and nitrogen-mustard treated rabbits.[15] Failure to find a tissue pyrogen in the blood stream is probably due to technical difficulties in dosage; the injection of a large quantity of leukocyte extract into the circulating blood of normal rabbits is followed by its complete disappearance from the circulation within less than two minutes.[16] Further studies of these tissue pyrogens are in progress.

The mechanism of action of bacterial pyrogens has been subjected to study by several groups of investigators during the past few years. It has been shown by Grant and Whalen[17] and by Atkins and Wood[18] that the febrile response to intravenously injected

bacterial pyrogen is accompanied by the appearance in the circulation of a fever-producing substance that differs from the original pyrogen in several important respects, including the fact that it will produce fever after a much-shortened latent period. Furthermore, Atkins and Wood[19] have demonstrated that this material is fully as active in producing fever in tolerant as in normal animals, that it does not appear in the blood of tolerant rabbits unless they have been given Thorotrast, and that the febrile response to this "endogenous pyrogen" is monophasic and of brief duration, resembling the fever produced by leukocyte extracts. Atkins and Wood point out the regular occurrence of severe neutropenia immediately after injection of bacterial pyrogen and suggest that injury to leukocytes and probably other cells with release of tissue pyrogens is the "preliminary step" during the latent period before fever begins. Other evidence supports the idea that endotoxins injure leukocytes.[20] Rabbits made neutropenic with nitrogen mustard are capable of responding to bacterial pyrogens with fevers as high as those of normal animals[21] although Stetson and Good[22] have shown clearly that the leukopenic response to endotoxins is an essential part of the mechanism whereby these substances are able to elicit the Shwartzman reaction in rabbit skin.

The development in this laboratory of a simple technique for permanent implantation of polyethylene catheters into the subarachnoid space of the rabbit has made feasible the investigation of the possible direct effect of bacterial pyrogen upon the central nervous system. The evidence for direct action on nervous tissue of endotoxins has recently been summarized by Thomas.[4] It was reported by Tschirgi[23] that intrathecal injection of tiny amounts of pyrogen produced fever in the dog after a latent period of only five minutes. Observations made in rabbits given single or multiple injections of endotoxin intrathecally can be summarized briefly.

1. Injection of endotoxin into the basilar cistern or other portions of the subarachnoid space is followed by high fever. Injection of physiologic saline, normal serum, or erythrocytes is without effect on body temperature.

2. To produce comparable fever, more than 4000 times the amount of endotoxin is required by the intravenous as by the intrathecal route.

3. Sustained rise in rectal temperature often begins less than two minutes after intrathecal injection of pyrogen. If the latent period is arbitrarily defined as the time required for rectal temperature to rise 1°F., the latent period is invariably 12 to 16 minutes shorter for intrathecal injection than for the intravenous route.

4. Tolerance acquired by daily *intravenous* injections of pyrogen exerts no influence upon the febrile response to intrathecal pyrogen and daily *intrathecal* injections have thus far produced no tolerance.

5. The febrile response to intrathecal pyrogen is prevented by amidopyrine and by cortisone, in appropriate dosage.

6. There is no immediate leukopenia after injection of pyrogen into the cerebrospinal fluid and thus far, no "endogenous" pyrogen has been demonstrable in the blood of animals made febrile by injection of endotoxin by this route.

7. The febrile response of rabbits to intrathecal injection of pyrogen is prolonged, lasting seven to eight hours in many instances, but to date, fever curves have invariably been monophasic; no secondary rises have been observed (in contrast to the usual biphasic fever curve after intravenous administration).

Although these results are preliminary, when taken with the findings of Atkins and Wood,[18, 19] they may be used as a basis for certain speculations. Bacterial pyrogens may act both *directly* and *indirectly* to produce fever. Such a dual mechanism might account for the biphasic fever curve in the rabbit. More rapid removal of injected pyrogen in tolerant animals could prevent tissue injury and lessen the portion of the fever produced by "endogenous" pyrogen although the central nervous system remains able to respond to exogenous or endogenous pyrogens. This train of events would explain several puzzling observations, including the "biphasic" fever curve as well as the ability of leukopenic rabbits to respond to pyrogen with

high fevers. Farr, Clark, Proffitt and Campbell[7] have pointed out that the lessening of the febrile response as animals become tolerant to pyrogens is first evidenced by disappearance of the secondary rise while it is only later that decrease in the first portion of the fever curve occurs. Finally, tolerance to pyrogens is never complete; animals continue to respond with low but distinct fevers even if daily injections are continued for many weeks. This persistent minimal febrile response could be explained by assuming that although tolerant animals eliminate injected pyrogen from the circulation rapidly enough to prevent tissue damage (and the "endogenous" pyrogen of Atkins and Wood fails to appear), enough of the injected pyrogen reaches the brain to exert a direct action upon nervous tissue which retains its sensitivity to this stimulus despite repeated exposures. These last suggestions are speculations and are indulged in only at the invitation of the Chairman of the Section on Microbiology. It is hoped that investigations now in progress will clarify some of the points that have been raised.

REFERENCES

1. Bennett, I. L., Jr. and Beeson, P. B. The properties and biologic effects of bacterial pyrogens, *Medicine 29*:365-400, 1950.
2. Wagner, R. R., Bennett, I. L., Jr. and LeQuire, V. S. The production of fever by influenzal viruses; factors influencing the febrile response to single injections of virus. *J. exp. Med. 90*:321-34, 1949.
3. Beeson, P. B. Alterations in body temperature, in *Principles of internal medicine*, 2.ed., New York, Blakiston Co., 1954, pp. 72-76.
4. Thomas L. The physiological disturbances produced by endotoxins, *Annu. Rev. Physiol. 16:* 467-90, 1954.
5. Beeson, P. B. Tolerance to bacterial pyrogens; factors influencing its development, *J. exp. Med. 86*:29-38, 1947.
6. Bennett, I. L., Jr. Observations on the fever caused by bacterial pyrogens; a study of the relationship between the fever caused by bacterial pyrogens and the fever accompanying acute infections, *J. exp. Med. 88*:267-78, 1948.
7. Farr, R. S., Clark, S. L., Proffitt, J. E. and Campbell, D. H. *Some humoral aspects of the development of tolerance to bacterial pyrogens in rabbits.* Research Report, Project NM-007081.12.02, Naval Medical Research Institute, Bethesda, Maryland, Aug. 14, 1953.
8. Beeson, P. B. Tolerance to bacterial pyrogens; role of the reticuloendothelial system, *J. exp. Med. 86*:39-44, 1947.
9. Braude, A. I., Carey, F. J., and Zalesky, M. Investigation of tolerance to bacterial endotoxin with radiochromium labelled E. coli endotoxin (abstract), *J. clin. Invest. 34*:923, 1955.
10. Bennett, I. L., Jr. and Beeson, P. B. Studies on the pathogenesis of fever; the effect of injection of extracts and suspensions of uninfected rabbit tissues upon the body temperature of normal rabbits, *J. exp. Med. 98*:447-92, 1953.
11. Bennett, I. L., Jr. and Beeson, P. B. Studies on the pathogenesis of fever; characterization of fever-producing substances from polymorphonuclear leukocytes and from the fluid of sterile exudates, *J. exp. Med. 98*:493-508, 1953.
12. Bennett, I. L., Jr. Studies on the pathogenesis of fever; failure to demonstrate fever-producing substances in the tissues of leukopenic rabbits, *Bull. Johns Hopkins Hosp. 98*:1-6, 1956.
13. Bennett, I. L., Jr. Studies on the pathogenesis of fever; the role of anaphylactic hypersensitivity. In preparation.
14. Bennett, I. L., Jr. Studies on the pathogenesis of fever; the fever accompanying pneumococcal infection in the rabbit, *Bull. Johns Hopkins Hosp.*, in press.
15. Bennett, I. L., Jr. Studies on the pathogenesis of fever; pneumococcal infection in leukopenic rabbits. In preparation.
16. Bennett, I. L., Jr. Studies on the pathogenesis of fever; further observations on the production of fever in rabbits by the fluid of sterile exudates, *Bull. Johns Hopkins Hosp. 98*:7-12, 1956.
17. Grant, R. and Whalen, W. J. Latency of pyrogen fever. Appearance of a fast-acting pyrogen in the blood of febrile animals and in plasma incubated with bacterial pyrogen, *Amer. J. Physiol. 173*:47-54, 1953.
18. Atkins, E. and Wood, W. B., Jr. Studies on the pathogenesis of fever; the presence of transferable pyrogen in the blood stream following the injection of typhoid vaccine, *J. exp. Med. 101*:519-28, 1955.
19. Atkins, E. and Wood, W. B., Jr. Studies on the pathogenesis of fever; identification of an endogenous pyrogen in the blood stream following the injection of typhoid vaccine, *J. exp. Med. 102*:499-516, 1955.
20. Berthrong, M. and Cluff, L. E. Studies of the effect of bacterial endotoxins on rabbit leucocytes; effect of intravenous injection of the substances with and without induction of the local Shwartzman reaction, *J. exp. Med. 98:* 331-47, 1953.
21. Bennett, I. L., Jr. and Cluff, L. E. Influence of nitrogen mustard upon reactions to bacterial endotoxins: Shwartzman phenomenon and fever, *Proc. Soc. exp. Biol. Med. 81*:304-07, 1952.
22. Stetson, C. A. and Good, R. A. Studies on the mechanism of the Shwartzman phenomenon; evidence for the participation of polymorphonuclear leucocytes in the phenomenon, *J. exp. Med. 93*:49-64, 1951.
23. Tschirgi, R. D. Influence of Piromen on central nervous system activity, *Proc. Fifth Piromen Conference*, Baxter Laboratories, Morton Grove, Illinois, 1952, p. 50-51.